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Public Comments on Developing an Unified Intercarrier Compensation:========

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Please do not eliminate switched telephone networks because they are essential to the 10 million Americans with electromagnetic sensitivities and because wireless systems affect peoples? health.

According to the Architectural and Transportation Barriers Compliance Board, an estimated 3% of the population (10 million people) have electromagnetic sensitivities (http://www.access-board.gov/research/ieq/intro.cfm). They cannot use wireless technology and have difficulty using computers. For them a switched telephone network is essential for voice service.

Medical research shows that wireless systems adversely affect humans. Three articles in respected medical journals show that cell phone use degrades men's sperm. Physicians at the Cleveland Clinic showed that the quality of semen in men degrades with less than 2 hours per day of cell use,(1) and that after 1 hour of exposure to cell phone radiation, the health of sperm in a test tube also degrades.(2) Researchers at the Australian Research Council detected DNA damage to sperm in a test tube after exposure to cell phone radiation.(3)

These results demonstrate that cell phones affect our bodies and makes me ask if there are other detrimental effects on men or women that are more difficult to detect. Also, how do cell phones affect our children?s health and their reproductive capabilities?

Two physicians found that people living in an area with a cell tower are four times more likely to get cancer than those who live in a similar area without a cell tower.(4) They compared cancer rates in two similar, neighboring villages before and after a cell tower was installed in one village.

Research that disputes these effects are typically funded by the telecom companies.(5)

Your support of switched telephone networks will aid people disabled by wireless exposure and preserve the health of all Americans.

References attached.

Source of Funding and Results of Studies of Health Effects of Mobile Phone Use: Systematic Review of Experimental Studies

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OBJECTIVES: There is concern regarding the possible health effects of cellular telephone use. We examined whether the source of funding of studies of the effects of low-level radiofrequency radiation is associated with the results of studies. We conducted a systematic review of studies of controlled exposure to radiofrequency radiation with health-related outcomes (electroencephalogram, cognitive or cardiovascular function, hormone levels, symptoms, and subjective well-being).

DATA SOURCES: We searched EMBASE, Medline, and a specialist database in February 2005 and scrutinized reference lists from relevant publications.

DATA EXTRACTION: Data on the source of funding, study design, methodologic quality, and other study characteristics were extracted. The primary outcome was the reporting of at least one statistically significant association between the exposure and a health-related outcome. Data were analyzed using logistic regression models.

DATA SYNTHESIS: Of 59 studies, 12 (20%) were funded exclusively by the telecommunications industry, 11 (19%) were funded by public agencies or charities, 14 (24%) had mixed funding (including industry), and in 22 (37%) the source of funding was not reported. Studies funded exclusively by industry reported the largest number of outcomes, but were least likely to report a statistically significant result: The odds ratio was 0.11 (95% confidence interval, 0.02–0.78), compared with studies funded by public agencies or charities. This finding was not materially altered in analyses adjusted for the number of outcomes reported, study quality, and other factors.

CONCLUSIONS: The interpretation of results from studies of health effects of radiofrequency radiation should take sponsorship into account.

KEY WORDS: electromagnetic fields, financial conflicts of interest, human laboratory studies, mobile phones. *Environ Health Perspect* 115:1–4 (2007). doi:10.1289/ehp.9149 available via *http://dx.doi.org/* [Online 15 September 2006]

The use of mobile telephones has increased rapidly in recent years. The emission of low-level radiofrequency electromagnetic fields leading to the absorption of radiation by the brain in users of handheld mobile phones has raised concerns regarding potential effects on health (Rothman 2000). However, the studies examining this issue have produced conflicting results, and there is ongoing debate on this issue (Ahlbom et al. 2004; Feychting et al. 2005). Many of the relevant studies have been funded by the telecommunications industry, and thus may have resulted in conflicts of interest (Thompson 1993). Recent systematic reviews of the influence of financial interests in medical research concluded that there is a strong association between industry sponsorship and pro-industry conclusions (Bekelman et al. 2003; Yaphe et al. 2001). This association has not been examined in the context of the studies of potential adverse effects of mobile phone use. We performed a systematic review and analysis of the literature to examine whether industry involvement is associated with the results and methodologic quality of studies.

Methods

We searched EMBASE (http://www.embase.com) and Medline (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed) in February 2005. Key and free text words included "cell(ular)," "mobile," "(tele)phone(s)" in connection with "attention," "auditory," "bioelectric," "brain physiology," "cardiovascular," "cerebral," "circulatory," "cognitive," "EEG," "health complaint(s)," "hearing," "heart rate," "hormone(s)," "learning," "melatonin," "memory," "neural," "neurological," "nervous system," "reaction," "visual," "symptom(s)," or "well-being." The search was complemented with references from a specialist database (ELMAR 2005) and by scrutinizing reference lists from the relevant publications. Articles published in English, German, or French were considered.

We included original articles that reported studies of the effect of controlled exposure with radiofrequency radiation on health-related outcomes ["human laboratory studies" in World Health Organization (WHO) terminology (Repacholi 1998)]. Health-related outcomes included electroencephalogram (EEG) recordings, assessments of cognitive or cardiovascular function, hormone levels, and subjective well-being and symptoms. We excluded studies of the risk of using mobile phones when driving a motor vehicle or operating machinery as well as studies on electromagnetic field (EMF) incompatibilities (e.g., pacemakers or hearing aids). Three of us (A.H., K.H., M.R.) independently extracted data on the source of funding (industry, public or charity, mixed, not reported) and potential confounding factors, including study design (crossover, parallel, other), exposure (frequency band, duration, field intensity, and location of antenna), and methodologic and reporting quality. Four dimensions of quality were assessed (Jüni et al. 2001; Repacholi 1998): *a*) randomized, concealed allocation of study participants in parallel or crossover trials; *b*) blinding of participants and investigators to allocation group; *c*) reporting of the specific absorption rate (SAR; watts per kilogram tissue) from direct measurement using a phantom head or three-dimensional dosimetric calculations ("appropriate exposure setting"); *d*) appropriate statistical analysis. For each item, studies were classified as adequate or inadequate/unclear.

The primary outcome was the reporting of at least one statistically significant ($p < 0.05$) association between radiofrequency exposure and a health-related outcome. The message in the title was also assessed. We distinguished among neutral titles [e.g., "Human brain activity during exposure to radiofrequency fields emitted by cellular phones" (Hietanen et al. 2000)], titles indicating an effect of radiation [e.g., "Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG" (Huber et al. 2000)], and titles stating that no effect was shown [e.g., "No effect on cognitive function from daily mobile phone use" (Besset et al. 2005)]. Finally, authors' declaration of conflicts of interest (present, absent) and affiliations (industry, other) were recorded. Differences in data extracted by A.H., K.H., and M.R. were resolved in the group, with the senior epidemiologist (M.R.) acting as the arbiter. In addition, two of us (K.H.M., M.E.), who were kept blind to funding

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The authors declare they have no competing financial interests.

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Figure 1. Identification of eligible studies.

Table 1. Characteristics of 59 experimental studies of the effects of exposure to low-level radiofrequency electromagnetic fields.

Study characteristic	Source of funding: Industry (n = 12)	Public or charity (n = 11)	Mixed (n = 14)	Not reported (n = 22)
Study design [no. (%)]				
Crossover trial	10 (83 3)	7 (63.6)	12 (85 7)	11 (50)
Parallel group trial	0 (0)	2 (18.2)	1 (7.1)	2 (9.1)
Other, unclear	2 (16 7)	2 (18.2)	1 (7 1)	9 (40.9)
Exposure [no. (%)]				
Location of antenna				
Next to ear	4 (33.3)	8 (72.7)	11 (78.6)	14 (63.6)
Other/unclear	8 (66.7)	3 (27 3)	3 (21.4)	8 (36 4)
Frequency band[a]				
900 MHz	11 (91.7)	8 (72.7)	13 (92.9)	14 (63.6)
Other frequencies	2 (16.7)	7 (63.6)	0 (0)	5 (22.7)
Unclear	0 (0)	0 (0)	1 (7.1)	5 (22.7)
Median duration of exposure (range)	180 (3–480)	20 (5–35)	45 (30–240)	30 (4–480)
Outcomes assessed [no. (%)][a]				
Electroencephalogram	7 (58 3)	5 (45.5)	8 (57 1)	12 (54.5)
Cognitive function tests	0 (0)	3 (27 3)	8 (57.1)	8 (36.4)
Hormone levels	5 (41 7)	0 (0)	0 (0)	2 (9.1)
Cardiovascular function	2 (16.7)	1 (9.1)	0 (0)	2 (9 1)
Well-being or symptoms	1 (8.3)	1 (9 1)	1 (7.1)	0 (0)
Other	4 (33.3)	3 (27.3)	1 (7.1)	3 (13 6)
Study quality [no. (%)][a]				
Randomization adequate	10 (83.3)	7 (63.6)	13 (92.9)	9 (40.9)
Participants and assessors blinded	1 (8.3)	3 (27.3)	8 (57 1)	3 (13.6)
SAR determined	4 (33.3)	4 (36 4)	8 (57.1)	2 (9.1)
Statistical analysis adequate	3 (25)	3 (27.3)	7 (50)	1 (4.5)
Median study size (range)	21 (8–38)	24 (13–100)	20 (13–96)	20 (8–78)

Percentages are column percentages.
[a]The same study could be listed in more than one category.

source, authors, and institutions, repeated extraction of data from abstracts and assessments of titles. Differences in data extracted by K.H.M. and M.E. were resolved with the senior epidemiologist (M.E.) acting as the arbiter. Based on the abstracts, we assessed whether authors interpreted their study results as showing an effect of low-level radiofrequency radiation, as showing no effect, or as indicating an unclear finding.

We used logistic regression models to assess whether the source of funding was associated with the reporting of at least one significant effect in the article (including the abstract). We examined the influence of potential confounders, such as the total number of outcomes that were reported in the article, the type of study (crossover, parallel, other), the four dimensions of study quality (adequate or not adequate/unclear), exposure conditions (position of the antenna next to the ear compared with other locations; use of the 900-MHz band compared with other bands; duration of exposure in minutes), as well as the type of outcome (e.g., cognitive function tests: yes vs. no). Variables were entered one at a time and, given the limited number of studies, models were adjusted for one variable only. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). All analyses were carried out in Stata (version 8.2; StataCorp., College Station, TX, USA).

Results

We identified 222 potentially relevant publications and excluded 163 studies that did not meet inclusion criteria (Figure 1). We excluded one study that had been funded by a company producing "shielding" devices that reduce EMF exposure (Croft et al. 2002). A total of 59 studies were included: 12 (20%) were exclusively funded by the telecommunications industry, 11 (19%) were funded by public agencies or charities, 14 (24%) had mixed funding (including industry and industry-independent sources), and in 22 (37%) studies the source of funding was not reported. None of 31 journals published a statement on possible conflicts of interest of the 287 authors listed in the bylines. Five (8%) studies had authors with industry affiliation. All studies except two (3%) were published in journals that use peer review, and one was published in a journal supplement. The bibliographic references are given in the Supplemental Material (http://www.ehponline.org/members/2006/9149/supplemental.pdf).

Blinded and open extraction of data yielded identical results with respect to the reporting of statistically significant effects in the abstract and the message of the title. Study characteristics are shown in Table 1. All studies were published during 1995–2005, with the number of publications increasing from one to

two publications per year to 11 publications in 2004. Median year of publication was 1998 for industry-funded studies, 2002 for public or charity funding and studies with mixed funding sources, and 2003 for studies that did not report their funding source. The median size of all the studies was small (20 study participants); most studies (n = 32, 54%) were of a crossover design and mimicked the exposure situation during a phone call, using the 900-MHz band with the antenna located close to the ear. Exposure duration ranged from 3 to 480 min, with a median of 33 minutes. Thirty-three (59%) studies measured outcomes during exposure, 14 (24%) postexposure, and 12 (20%) at both times. Thirty-nine (66%) studies prevented selection bias with adequate randomization; 15 (25%) blinded both participants and assessors; in 18 (31%) the field intensity had been assessed appropriately, with SAR values ranging from 0.03 to 2 W/kg tissue. Finally, in 14 (24%) studies we considered the statistical analysis to be adequate. Study quality varied by source of funding: Studies with mixed funding (including public agencies or charities and industry) had the highest quality, whereas studies with no reported source of funding did worst (Table 1).

Forty (68%) studies reported one or more statistically significant results ($p < 0.05$) indicating an effect of the exposure (Table 2). Studies funded exclusively by industry reported on the largest number of outcomes but were less likely to report statistically significant results: The OR for reporting at least one such result was 0.11 (95% CI, 0.02–0.78), compared with studies funded by public agencies or charities (Table 3). This finding was not materially altered in analyses adjusted for the number of outcomes reported, study design and quality, exposure characteristics, or outcomes [Table 3; see Supplemental Material, Table 1 (http://www.ehponline.org/members/2006/9149/supplemental.pdf)]. Similar results were obtained when restricting analyses to results reported in abstracts (OR = 0.29; 95% CI, 0.05–1.59) or on the conclusions in the abstract (OR = 0.10, 95% CI, 0.009–1.10). Thirty-seven (63%) studies had a neutral title, 11 (19%) a title reporting an effect, and 11 (19%) a title reporting no effect (Table 2).

Discussion

We examined the methodologic quality and results of experimental studies investigating the effects of the type of radiofrequency radiation emitted by handheld cellular telephones. We hypothesized that studies would be less likely to show an effect of the exposure if funded by the telecommunications industry, which has a vested interest in portraying the use of mobile phones as safe. We found that the studies funded exclusively by industry were indeed substantially less likely to report statistically significant effects on a range of end points that may be relevant to health.

Our findings add to the existing evidence that single-source sponsorship is associated with outcomes that favor the sponsors' products (Bekelman et al. 2003; Davidson 1986; Lexchin et al. 2003; Stelfox et al. 1998). Most previous studies of this issue were based on studies of the efficacy and cost-effectiveness of drug treatments. A recent systematic review and meta-analysis showed that studies sponsored by the pharmaceutical industry were approximately four times more likely to have outcomes favoring the sponsor's drug than studies with other sources of funding (Lexchin et al. 2003). The influence of the tobacco industry on the research it funded has also been investigated (Barnes and Bero 1996, 1998; Bero 2005). To our knowledge, this is the first study to examine this issue in the context of exposure to radiofrequency electromagnetic fields.

Our study has several limitations. We restricted our analysis to human laboratory studies. This resulted in a more homogenous set of studies, but may have reduced the statistical power to demonstrate or exclude smaller associations. The WHO has identified the need for further studies of this type to clarify the effects of radiofrequency exposure on neuroendocrine, neurologic, and immune systems (Foster and Repacholi 2004). We considered including epidemiologic studies but found that practically all of them were publicly funded. The study's primary outcome—the reporting of statistically significant associations—is a crude measure that ignores the size of reported effects. However, we found the same trends when assessing the authors' conclusions in the abstracts.

Although we have shown an association between sponsorship and results, it remains unclear which type of funding leads to the most accurate estimates of the effects of

Table 2. Results from assessments of article text, abstract, and title of 59 experimental studies of the effects of exposure to low-level radiofrequency electromagnetic fields.

	Source of funding			
	Industry (n = 12)	Public or charity (n = 11)	Mixed (n = 14)	Not reported (n = 22)
Article text				
No. (%) of studies with at least one result suggesting an effect at $p < 0.05$	4 (33)	9 (82)	10 (71)	17 (77)
Median no. (range) of outcomes reported	17.5 (4–31)	10 (1–80)	16 (9–44)	7 (1–35)
Median no. (range) of outcomes suggesting an effect at $p < 0.05$	0 (0–6)	1.5 (0–7)	3 (0–15)	1.5 (0–12)
Abstract[a]	(n = 12)	(n = 11)	(n = 14)	(n = 20)
No. (%) of studies with at least one result suggesting a significant effect	4 (33)	7 (64)	10 (71)	15 (75)
Median no. (range) of outcomes reported	3.5 (1–36)	3 (1–5)	6 5 (3–44)	3 (1–64)
Median no. (range) of outcomes suggesting a significant effect	0 (0–6)	1 (0–3)	2 (0–5)	1.5 (0–7)
Authors' interpretation of results [no. (%)]				
No effect of radiofrequency radiation	10 (83.3)	5 (45.5)	4 (28.6)	5 (22.7)
Effect of radiofrequency radiation	1 (8.3)	5 (45 5)	8 (57.1)	14 (63.6)
Unclear finding	1 (8.3)	1 (9)	2 (14.3)	3 (13.6)
Title [no. (%)]				
Neutral	7 (58)	5 (46)	8 (57)	17 (77)
Statement of effect	0 (0)	4 (36)	3 (21)	4 (18)
Statement of no effect	5 (42)	2 (18)	3 (21)	1 (5)

Percentages are column percentages.
[a]Two publications that did not report their source of funding had no abstracts.

Table 3. Probability of reporting at least one statistically significant result ($p < 0.05$) according to source of funding: crude and adjusted ORs (95% CIs) from logistic regression models.

	Source of funding				
	Industry (n = 12)	Public or charity (n = 11)	Mixed (n = 14)	Not reported (n = 22)	p-Value[a]
Crude	0.11 (0.02–0 78)	1 (reference)	0.56 (0 08–3.80)	0.76 (0.12–4.70)	0.04
Adjusted for					
No of reported outcomes	0.12 (0 02–0.89)	1 (reference)	0.60 (0.08–4.28)	0.96 (0.15–6.23)	0.04
Median study size	0.08 (0.009–0.62)	1 (reference)	0.61 (0.08–4.59)	0.57 (0.08–4.02)	0.02
Study design (crossover, parallel, or other)	0 08 (0.01–0.68)	1 (reference)	0 38 (0.05–3.07)	1.16 (0.16–8.61)	0.029
Study quality					
Randomization adequate	0.04 (0–0.56)	1 (reference)	0.16 (0.01–2.15)	1.27 (0.16–9.89)	0 005
Participants and assessors blinded	0.14 (0 02–0 96)	1 (reference)	0.54 (0 08–3.91)	0.76 (0.12–4.8)	0.09
Statistical analysis adequate	0.12 (0 02–0.85)	1 (reference)	0 67 (0.09–4.85)	0.54 (0.08–3.76)	0.07
Exposure setting appropriate	0.13 (0.02–0.89)	1 (reference)	0 47 (0.07–3 39)	0.86 (0.14–5.5)	0.06

Models adjusted for one variable at a time.
[a]From likelihood ratio tests.

radiofrequency radiation. For example, if researchers with an environmentalist agenda are more likely to be funded by public agencies or charities, then their bias may result in an overestimation of effects. Interestingly, studies with mixed funding were of the highest quality. The National Radiological Protection Board (NRPB 2004) reviewed studies of health effects from radiofrequency (RF) fields and concluded that "scientific evidence regarding effects of RF field exposure from mobile phones on human brain activity and cognitive function ... has included results both supporting and against the hypothesis of an effect." We found that the source of funding explains some of the heterogeneity in the results from different studies. The association was robust and little affected by potential confounding factors such as sample size, study design, or quality.

Possible explanations for the association between source of funding and results have been discussed in the context of clinical research sponsored by the pharmaceutical industry (Baker et al. 2003; Bekelman et al. 2003; Lexchin et al. 2003). The association could reflect the selective publication of studies that produced results that fitted the sponsor's agenda. Sponsors might influence the design of the study, the nature of the exposure, and the type of outcomes assessed. In multivariate logistic regression analysis, the only factor that strongly predicted the reporting of statistically significant effects was whether or not the study was funded exclusively by industry. We stress that our ability to control for potential confounding factors may have been hampered by the incomplete reporting of relevant study characteristics.

Medical and science journals are implementing policies that require authors to disclose their financial and other conflicts of interest. None of the articles examined here included such a statement, in line with a survey of science and medical journals that showed that adopting such policies does not generally lead to the publication of disclosure statements (Krimsky and Rothenberg 2001). A review of 2005 instructions to authors showed that 15 (48%) of the 31 journals included in our study had conflict of interest policies. Our results support the notion that disclosure statements should be published, including statements indicating the absence of conflicts of interest. The role of the funding source in the design, conduct, analysis, and reporting of the study should also be addressed.

There is widespread concern regarding the possible health effects associated with the use of cellular phones, mobile telephone base stations, or broadcasting transmitters. Most (68%) of the studies assessed here reported biologic effects. At present it is unclear whether these biologic effects translate into relevant health hazards. Reports from national and international bodies have recently concluded that further research efforts are needed, and dedicated research programs have been set up in the United States, Germany, Denmark, Hungary, Switzerland, and Japan. Our study indicates that the interpretation of the results from existing and future studies of the health effects of radiofrequency radiation should take sponsorship into account.

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INCREASED INCIDENCE OF CANCER NEAR A CELL-PHONE TRANSMITTER STATION.

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Increased Incidence of Cancer near a Cell-Phone Transmitter Station
by Ronni Wolf and Danny Wolf

Abstract

Significant concern has been raised about possible health effects from exposure to radiofrequency (RF) electromagnetic fields, especially after the rapid introduction of mobile telecommunications systems. Parents are especially concerned with the possibility that children might develop cancer after exposure to the RF emissions from mobile telephone base stations erected in or near schools. The few epidemiologic studies that did report on cancer incidence in relation to RF radiation have generally presented negative or inconsistent results, and thus emphasize the need for more studies that should investigate cohorts with high RF exposure for changes in cancer incidence. The aim of this study is to investigate whether there is an increased cancer incidence in populations, living in a small area, and exposed to RF radiation from a cell-phone transmitter station.

This is an epidemiologic assessment, to determine whether the incidence of cancer cases among individuals exposed to a cell-phone transmitter station is different from that expected in Israel, in Netanya, or as compared to people who lived in a nearby area. Participants are people (n=622) living in the area near a cell-phone transmitter station for 3-7 years who were patients of one health clinic (of DW). The exposure began 1 year before the start of the study when the station first came into service. A second cohort of individuals (n=1222) who get their medical services in a clinic located nearby with very closely matched, environment, workplace and occupational characteristics was used for comparison.

In the area of exposure (area A) eight cases of different kinds of cancer were diagnosed in a period of only one year. This rate of cancers was compared both with the rate of 31 cases per 10,000 per year in the general population and the 2/1222 rate recorded in the nearby clinic (area B). Relative cancer rates for females were 10.5 for area A, 0.6 for area B and 1 for the whole town of Netanya. Cancer incidence of women in area A was thus significantly higher ($p<0.0001$) compared with that of area B and the whole city. A comparison of the relative risk revealed that there were 4.15 times more cases in area A than in the entire population.

The study indicates an association between increased incidence of cancer and living in proximity to a cell-phone transmitter station.

Key Words:

Radiofrequency radiation; Cell-phone transmitter station (cell-phone antenna); Cancer incidence study; Netanya.

Introduction

Much concern has been expressed about possible health effects from exposure to radiofrequency (RF) electromagnetic fields, particularly following publication of scientific reports suggesting that residence near high voltage power lines may be associated with an increased risk of developing childhood leukemia. While interest tended to focus on microwave ovens and radar equipment in the past, it is now mobile telecommunication that attracts the most attention. The rapid introduction of mobile telecommunications systems, the exponential increase in the use of such phones, and the many base stations needed for serving them have engendered renewed concerns about exposure to RF radiation.

The biological effects of low level electromagnetic fields and a possible potential relation to cancer causation are controversial. There have been several epidemiological studies of the possible adverse health effects associated with environmental exposure to extremely low frequency (0-300 Hz) non-ionizing radiation, such as that emitted by power cables and electric substations, linking such exposure to leukemia, brain cancer, male breast cancer and skin and eye melanoma (1-11).

Far less attention has been paid to health hazards from environmental exposure to radiation in the RF range (100 kHz to 300 GHz), including the radiation emitted from cell-phone equipment, in the frequencies of 850 MHz, at field strengths much below those required to produce thermal effects. The few epidemiologic studies that did report on cancer incidence in relation to RF radiation (mainly from occupational exposure including microwave and radar and from living in proximity to TV towers) have generally presented negative or inconsistent results, or were subject to possible confounding from other exposures (12-20).

Laboratory studies in this area have also been confusing and conflicting. While some animal studies suggested that RF fields accelerate the development of cancers, other studies found no carcinogenic effect (21).

Obviously, there is an urgent need for extensive, well-conducted epidemiological and laboratory studies (21-24).

An opportunity for studying the effect of RF radiation presented itself in South Netanya, where a cell-phone transmitter station was located in the middle of a small area. We took advantage of the fact, that most of the population in the investigated area belong to one outpatient clinic (of DW), and undertook an epidemiologic assessment, in which we compared the cancer incidence of this area to those of a nearby clinic, to the national incidence rates of the whole country and to the incidence rates in the whole town of Netanya.

Material and methods

Radio-frequency radiation

The cell-phone transmitter unit is located at the south of the city of Netanya in an area called Irus (area A). It first came into service in 7/96. The people in this area live in half a circle with a 350 meter radius centered on the transmitter.

The antenna is 10 meters high. The antenna bears total maximum transmission power at frequencies of 850 MHz of 1500 watt when working at full power.

Both measured and predicted power density (for the frequencies of 850 MHz) in the whole exposed area were far below 0.53 $\mu w/cm^2$ thus the power density is far below the current guidelines which are based on *the thermal effects of RF exposure.* Exact measured power density in each house are described in table 1.

The current Israeli standard uses 50 packets/sec with Time-Division-Multiple-Access (TDMA) quadrature modulation. The antenna produces 50 packets/sec, using a 3:1 multiplexed Time-Division-Multiple-Access (TDMA) modulation with a 33% duty cycle.

Statistical analysis:

We conducted a cancer incidence study to investigate the incidence of cancer cases of individuals exposed to a cell-phone transmitter station, in comparison to those of a nearby clinic, to the national incidence rates of the whole country and to the incidence rates in the whole town of Netanya.

The cohort included 622 people living in the Irus area (area A) for at least 3-7 years and were patients of one health clinic (of DW). The exposure began in 7/96 which was 1 year before the start of our study.

Statistical analysis was based on the comparison of observed and expected numbers of cancer cases.

In order to compare incidence rates, 95% confidence intervals were computed.

The observed number of cancer cases is the number of all the cancer cases in the exposed cohort in the period between 7/97 - 6/98.

In order to estimate relative risk, rate ratios were computed using the rate of 3 different cohorts as the base (the expected values):

The rate in a nearby clinic (which serves a population of 1222 people, all of them living in area B) during the same period of time, i.e. 7/97 - 6/98. In order to compare area A and area B populations we used:

χ^2 test to compare origin and sex division

t- test to compare age means

The national incidence rates of the whole country.

The incidence rates in the whole town of Netanya where the 2 clinics (of area A and B) are located. The data of 2 and 3 were given to us by the Israel cancer registry and are updated to the years 91-94.

We also examined the history of the exposed cohort (of the A area) for malignancies in the 5 years before the exposure began and found only 2 cases in comparison to 8 cases detected one year after the transmitter station came into service.

Results

Of the 622 people of area A, eight cases of different kinds of cancer were diagnosed in a period of only one year (from July 1997 to June 1998). Details on these cases are presented in Table 1. Briefly, we found 3 cases of breast carcinoma, and one case of ovary carcinoma, lung carcinoma, Hodgkin's disease, osteoid osteoma, and hypernephroma.

This rate of cancers in the population of area A was compared both with the rate of 31 cases per 10,000 per year in the general population and the 2/1222 rate recorded in a nearby clinic. To each one of the rates, a 95 percent confidence interval was calculated (Table 2): the rates in area A were significantly higher than both those in area B, and the population as a whole.

A comparison of the relative risk revealed that there were 4.15 times more cases in area A than in the entire population.

The population characteristics of areas A and B were very similar (Table 2-5). The χ^2 test for comparing gender and origin frequencies showed no significant differences in these parameters between the two areas. Age means, as compared by t-test and age distribution stratum also showed no significant difference between the two groups.

Table 2a lists the rates of cancer incidence of areas A and B compared to data of the whole town of Netanya. The comparison clearly indicated that the cancer incidence of women in area A is significantly higher ($p<0.0001$) compared with that of the whole city.

Discussion

Our study indicates an association between an increased incidence of cancer and living in proximity to a cell-phone transmitter station.

Studies of this type are prone to biases. Possible methodological artefacts to explain our alarming results were considered:

Differences in socioeconomic class and employment status, and demographic heterogeneity due to differences in age, sex and ethnicity were excluded. The two areas that were compared have very closely matched environment, workplace and occupational characteristics.

Confounding variables affecting individuals could not be absolutely adjusted for, however, there was no ionizing radiation that could affect the whole community except the previously mentioned mobile antenna station. There is no traffic density in this area, neither is there any industry or any other air pollution. The population of area A

(on which adequate data could be gathered) did not suffer from uncommon genetic conditions, nor did they receive carcinogenic medications.

Differences in diagnosis and registration of cancer cases. Although we cannot altogether exclude the possibility that higher awareness of the physician responsible for area A led to an artificial increase in cancer cases in this area, this possibility seems to us very unlikely, since both are qualified family physicians.

Several findings are of particular interest:

The measured level of RF radiation (power density) in the area was low; far below the current guidelines based on the thermal effects of RF exposure. We suggest, therefore, that the current guidelines be re-evaluated.

The enormous short latency period; less than 2 years, indicates that if there is a real causal association between RF radiation emitted from the cell-phone base station and the cancer cases (which we strongly believe there is), then the RF radiation should have a very strong promoting effect on cancer at very low radiation!

Although the possibility remains that this clustering of cancer cases in one year was a chance event, the unusual sex pattern of these cases, the 6 different cancer kinds, and the fact that only one patient smoked make this possibility very improbable and remote. It should be noted that 7 out of 8 cancer cases were women, like in the work of Maskarinec (25) who found 6 out of 7 leukemia cases in proximity to radio towers to occur in girls. Such unusual appearances of cancer cases due to one accused factor on two completely different occasions is alarming.

We are aware of at least 2 areas in which a drastic increase in the incidence of cancer cases occurred near a cell-phone antenna, however, the setup was not suitable for a well design study of those cases. In one of them (which also got publication in the daily newspapers) there were 6 out of 7 cancer cases in women working in a store in close proximity to a cell-phone antenna.

In conclusion, the results of this study showed that there was a significantly greater incidence of cancers of all kinds within the vicinity of a cell-phone transmitter station.

It would be certainly too premature to draw any conclusions from our results before they are confirmed and repeated by other studies from other areas, particularly in view of the fact that a great majority of papers on this subject showed that RF fields and mobile telephone frequencies were not genotoxic, did not induce genetic effects in vitro and in vivo, and were not found to be teratogenic or to induce cancers (24). The results of this paper should, however, serve as an alarm and emphasize the need for further investigations.

Addendum

At one year following the close of the study, 8 new cases of cancer were diagnosed in area A and two cases in area B. Among the cases diagnosed in area A was one of osteoid osteoma, the second case from the beginning of the study.

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Table 1: Cancer cases in area A

NAME	AGE	SEX	ORIGIN[1]	SMO-KING	CANCER TYPE	Measured power density in $\mu w/cm^2$
Hemda	52	f	ash	No	Ovary ca stage 1	$0.3\mu w/cm^2$
Edna	42	f	sph	No	Breast ca in situ	$0.4\mu w/cm^2$
Tania	54	f	ash	No	Breast ca	$0.5\mu w/cm^2$
Neli	67	f	ash	Yes	Breast ca	$0.4\mu w/cm^2$
Galit	24	f	ash	No	Hodgkins	$0.5\mu w/cm^2$
Miriam	61	f	sph	No	Lung ca	$0.3\mu w/cm^2$
Masal	37	f	sph	No	Osteoid osteoma	$0.4\mu w/cm^2$
Max	78	m	ash	No	Hypernephroma	$0.3\mu w/cm^2$

1. Origin: ash - Ashkenazien Jews sph - Spharadic Jews

Table 2: Cancer rates in area A, B and the total population.

	No. of cancer cases	populati on size	Rate per year per 10,000	confide interval lower limit	ce (95%) upper limit	relative risk
Area A	8	622	129	40.1	217.2	4.15
Area B	2	1222	16	-6.3	39.0	0.53
total populat	31	10,000	31	20.1	41.9	1.00

Table 2a: Cancer rates in area A, B and the whole town.

	Male		Female	
	rate	Relative rate	rate	relative rate
Area A	33	1.4	262	10.5
Area B	17	0.7	16	0.6
Whole town	24	1	25	1

Table 3: Comparing area A to area B by gender.

Gender	Area A		Area B	
	N	%	N	%
male	290	49	669	49
female	305	51	685	51

Table 4: Comparing area A to area B by origin.

Origin	Area		Area	
	N	%	N	%
Sfaradic	340	55	551	45
Ashkenaz	239	38	620	51
Russian	41	7	51	4

Table 5: Comparing age means in both areas.

	Area A		Area B	
	mean	Std	mean	std
age	26.5	17.9	25.5	12.4

Table 5: Age distribution by stratum.

	0-1	**1-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**>70**
IRUS	16	143	157	65	70	88	41	21	21
POLEG	31	285	257	139	180	158	83	55	34

Mobile Phone Radiation Induces Reactive Oxygen Species Production and DNA Damage in Human Spermatozoa *In Vitro* complete 2009 7

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Mobile Phone Radiation Induces Reactive Oxygen Species Production and DNA Damage in Human Spermatozoa *In Vitro*
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Abstract

Background

In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro.

Principal Findings

Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were

significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P<0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure.

Conclusions

RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

- **Other Sections▼**

Introduction

Male infertility is a distressingly common condition affecting about 1 in 20 of the male population [1]. In a majority of cases, the male partner produces sufficient numbers of spermatozoa to achieve fertilization but there are functional defects in these cells that prevent conception from occurring [2]. Despite several decades of research, the causes of such functional deficiencies in human spermatozoa remain largely unresolved. However, one contributory factor that has recently emerged is the quality of the sperm DNA delivered to the oocyte at the moment of fertilization [3]. Fragmentation of DNA in the male germ line has been associated with impaired fertilization, poor embryonic development, high rates of miscarriage and an increased incidence of morbidity in the offspring, including childhood cancer [3], [4]. In view of the seriousness of these clinical outcomes, attention has recently focused on the environmental and genetic factors that might be involved in the aetiology of DNA damage in the male germ line.

These investigations have suggested that one of the environmental factors potentially involved in the etiology of DNA damage in human spermatozoa is an increased exposure to radio-frequency electromagnetic radiation (RF-EMR) emitted from mobile phones. This association was initially suggested by an epidemiological study which found negative correlations between mobile phone usage and various attributes of semen quality, particularly motility [5]. This was immediately followed by an experimental study involving exposure of male mice to RF-EMR, which revealed a significant impact on the integrity of both the mitochondrial and nuclear genomes [6]. Recently, the negative impact of mobile phone usage on semen quality in human males was confirmed in a study that found the duration of exposure to be correlated with defects in sperm count, motility, viability, and normal morphology [7]. In light of these data, there is now an urgent need to determine whether exposure of human spermatozoa to RF-EMR can also induce DNA damage and to resolve the cellular mechanisms involved.

Several studies have found an association between human health and exposure to RF-EMR, with emphasis on a range of clinical conditions including childhood leukaemia, brain tumours, genotoxicity and neurodegenerative disease [8], [9]. While the cellular mechanisms underpinning these effects have not been completely resolved, it has been suggested that oxidative stress could be a key factor [10]. However, extensive analysis of the importance of oxidative stress in mediating the pathological effects of RF-EMR has generated conflicting results, possibly due to differences in the fundamental redox

susceptibility of the cell lines employed in these analyses [11]. In this context, it is significant that human spermatozoa are uniquely sensitive to oxidative stress for a variety of reasons. Firstly, these cells are largely devoid of the cytoplasm that in somatic cells houses the antioxidant enzymes that offer a first line of defense against free radical attack [12]. Secondly, these cells possess abundant targets for the induction of peroxidative damage including polyunsaturated fatty acids and DNA[12]–[14]. Thirdly, these cells are professional generators of reactive oxygen species, that appear to emanate largely from the sperm mitochondria and, possibly, plasma membrane NAD(P)H oxidases [15], [16]. Thus if any cell type would be vulnerable to the oxidative stress reportedly generated on exposure to RF-EMR, it would be human spermatozoa.
In light of these considerations, we have conducted a careful analysis of the biological consequences of exposing human spermatozoa to RF-EMR. The study design involved overnight exposure to RF-EMR at a defined frequency (1.8 GHz), over a range of SAR values that both covered the emission characteristics of mobile phones and generated sufficient dose-response data to shed light on the underlying pathophysiological mechanisms. Moreover, the temperature of the incubations was maintained at 21°C to avoid any secondary heating effects. The results clearly demonstrate that exposure to this type of radiation not only stimulates free radical generation by the sperm mitochondria but also creates a state of oxidative stress characterized by the formation of oxidative base adducts and DNA fragmentation. These data clearly have important implications for the safety of mobile phone use and highlight the potential importance of RF-EMR in the etiology of male infertility and childhood disease.

- **Other Sections▼**

Results

RF-EMR disrupts human sperm motility and vitality and induces intracellular reactive oxygen species (ROS) production

In an initial experiment, functional human spermatozoa isolated from the high density region of Percoll gradients and suspended in BWW medium were exposed to RF-EMR at an SAR of 27.5 W/kg. This exposure induced a highly significant decline in both vitality ($p<0.001$; Figure 1A) and motility ($p<0.01$; Figure 1B) compared with the unexposed controls. Exposed spermatozoa also produced significantly higher amounts of ROS than background levels as measured by both the dihydroethidium (DHE) ($p<0.001$; Figure 1C) and MitoSOX red (MSR) probes ($p<0.001$; Figure 1D) suggesting that free radical generation had been initiated as a consequence of RF-EMR and that the mitochondria were significantly involved in this response.

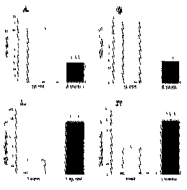

Figure 1
RF-EMR exposure decreases motility and vitality of human sperm while also inducing intracellular ROS.

RF-EMR has a negative impact on human spermatozoa over a range of SAR values

In light of these results we then extended the range of SAR values over which the consequences of RF-EMR radiation were examined (0.4 W/kg–27.5 W/kg) to include the values covered by conventional mobile phones (0.5 W/kg–1.5 W/kg).

High quality spermatozoa selected in discontinuous Percoll gradients displayed a decline in both vitality and motility after exposure to RF-EMR in a dose- dependent manner. The control populations maintained an average vitality of 89%; however, significant reductions in vitality were observed at exposure levels as low as 1.0 W/kg ($p<0.01$) (Figure 2A). Similarly, the control populations maintained motilities at an average of 86% over the incubation period, however after exposure to RF-EMR at levels of 1.0 W/kg, motility was observed to significantly decrease to 68% ($p<0.05$) and decreased still further at higher SAR exposures (Figure 2B).

Figure 2
RF-EMR exposure reduces motility and vitality of human spermatozoa, in an SAR dependent manner.

Reactive Oxygen Species are central to the RF-EMR response

Exposure of human spermatozoa to RF-EMR over a range of SAR levels resulted in a dose-dependent activation of ROS generation, as detected by the DHE probe (Figure 3A). In this analysis, a significant increase in ROS positive cells was observed after exposure at 1.0 W/kg ($p<0.05$); thereafter ROS production rose rapidly with SAR values up to 4.3 W/kg and then began to plateau reaching a peak of 30% at the highest exposure levels assessed (Figure 3A). To determine whether such increases in ROS production might originate from the sperm mitochondria, MSR was employed as a probe. Spermatozoa exposed to increasing levels of RF-EMR, generated a significant, dose-dependent increase in ROS generation by the mitochondria. The response rose rapidly following RF-EMR exposure reaching statistical significance ($p<0.001$) at an SAR value 2.8 W/kg at which point 16% of the exposed cells were MSR positive. At SAR values above 4.3 W/kg, RF-EMR induced mitochondrial ROS begun to plateau reaching 30% at the maximal SAR values assessed (Figure 3B). By plotting the DHE positive cells against the MSR response for the entire data set (Figure 3D) we observed an extremely strong correlation ($R^2 = 0.823$) between these signals, suggesting that a majority of the ROS production elicited by RF-EMR involved electron leakage from the mitochondrial electron transport chain.

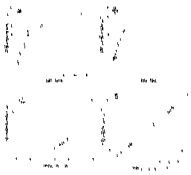

Figure 3
RF-EMR induces ROS generation in human spermatozoa, in an SAR-dependent manner unrelated to thermal effects.

In order to control for bulk thermal effects of RF-EMR exposure, spermatozoa were also incubated at temperatures ranging from 21°C–50°C for 2 h (Figure 3C). This analysis did reveal an effect of heat on free radical generation by human spermatozoa possibly due to the activation of an apoptotic response, however these effects were only significant above 40°C. Thus at the temperature at which these experiments were performed (21°C) the highest observed RF-EMR-induced temperature rise (+0.4°C at 27.5 W/kg), could not of itself account for the increased ROS response observed across the range of SAR settings evaluated in this study.

RF-EMR induces oxidative DNA damage (8-OH-dG)

In order to determine whether the ROS generation induced on exposure of human spermatozoa to RF-EMR resulted in a state of oxidative stress, we monitored the expression of 8-hydroxy-2′-deoxyguanosine (8-OH-dG), a marker for oxidative damage to sperm DNA. As the SAR level was increased, the amount of oxidative DNA damage expressed in the spermatozoa became elevated (Figure 4A). A significant increase in 8-OH-dG expression became apparent at low SAR values (<5.0 W/kg) rising to a maximum of around 20% at the highest levels of exposure (27.5 W/kg). By plotting the 8-OH-dG positive cells against the MSR signal (Figure 4B) it was apparent that a strong positive correlation existed between the two parameters ($R^2 = 0.727$); the higher the level of mitochondrial ROS generation, the greater the degree of oxidative DNA damage in the spermatozoa.

Figure 4
RF-EMR induces oxidative DNA damage in human spermatozoa.

RF-EMR induces DNA fragmentation in human spermatozoa

To determine whether the oxidative DNA base damage precipitated by RF-EMR-induced ROS generation had any impact on DNA stand breaks in human spermatozoa, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was utilized. As illustrated in Figure 5A, human spermatozoa responded to RF-EMR exposure, with a significant increase in DNA strand breaks at an SAR of 2.8 W/kg ($p<0.05$) that increased rapidly with rising SAR values and then reached a plateau so that at the highest SAR level assessed (27.5 W/kg), 29% of the cells expressed significant DNA fragmentation. This DNA damage was highly correlated with free radical generation by the sperm mitochondria giving a correlation coefficient of $R^2 = 0.861$ (Figure 5B). Moreover, the level of DNA fragmentation was highly correlated with 8-OH-dG formation ($R^2 = 0.725$; Figure 5C) such that sperm cells exhibiting high levels of oxidative DNA damage, also possessed high levels of DNA fragmentation.

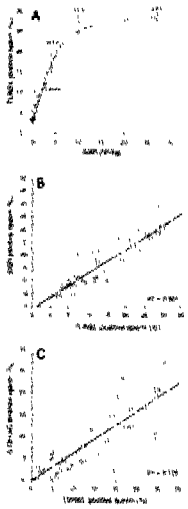

Figure 5
RF-EMR induces DNA fragmentation in human spermatozoa.

- **Other Sections▼**

Discussion

While a high proportion of the male population suffers from infertility associated with defective sperm function [17], the etiology of this condition remains largely unresolved. Notwithstanding the general paucity of information in this area, recent studies have highlighted the interesting finding that male infertility patients are frequently characterized by high levels of DNA damage to their spermatozoa[18]. In light of these data, we have hypothesized that the disruption of sperm fertilizing potential and the concomitant presence of high levels of DNA damage in the sperm nucleus involves a common causative mechanism in the form of oxidative stress [19].

Oxidative stress has been known for some time to limit the fertilizing potential of human spermatozoa through the induction of peroxidative damage to the sperm plasma membrane [13], [20]. Oxidative stress is also known to be associated with DNA damage in human spermatozoa [21]. Furthermore, the source of the free radicals responsible for generating such stress appears to be the mitochondria[15]. However, the factors responsible for inducing the mitochondria to leak electrons and propagate the production of ROS have not been elucidated. The research described in this article suggests that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high levels of ROS, might be excess exposure to RF-EMR from sources such as mobile phones.

In a pilot study, human spermatozoa were found to respond to RF-EMR (at 1.8 GHz with a SAR of 27.5 W/kg) with a range of negative changes including dramatic declines in both sperm vitality and motility. We also observed significant increases in both cytoplasmic ROS levels (DHE) as well as mitochondrial ROS levels (MSR) after RF-EMR exposure. We have previously shown that the chemical induction of mitochondrial ROS production with rotenone can precipitate a state of oxidative stress leading to high levels of lipid peroxidation and a loss of sperm motility [15]. Therefore, these data highlight the particular vulnerability of human spermatozoa to oxidative attack and the potential significance of sperm mitochondria in the generation of free radicals.

To assess whether similar effects could be observed at lower power densities, closer to the SAR values associated with mobile phones (0.5–1.5 W/kg) a dose-dependent analysis was conducted. In addition to the conventional assessments of motility and vitality, assays were included to assess the potential for RF-EMR to induce sperm DNA damage and further, whether the DNA damage was oxidative in nature. Confirmation of the detrimental effects of RF-EMR on human sperm was again observed. Over the power density range employed, a significant ($P<0.001$) dose-dependent response for all sperm parameters was observed, including motility, vitality, ROS generation by the whole cell, ROS generation by the mitochondria, oxidative DNA damage and DNA fragmentation. Furthermore, the profiles of all the observed effects with respect to SAR were intriguingly similar, suggesting a common underlying mechanism.

Specifically, all of the responses examined showed an extremely rapid change at low SAR exposures that then reached a plateau at a point where around 30% of the sperm population was affected. This suggests that while we were careful to use only Percoll-purified, high quality spermatozoa in this analysis, there exists within this cell population, a cohort of spermatozoa that are particularly vulnerable to the induction of oxidative stress by RF-EMR. These spermatozoa may have compromised mitochondria, poorly

remodeled chromatin or a combination of such factors [15], [22]. Heterogeneity within the sperm population is a feature of the human condition. However, this does not mean that a majority of spermatozoa would not, ultimately, be affected by RF-EMR in vivo; much would depend on the duration of exposure. In vitro, we are limited by the inability of human spermatozoa to survive for more than 24 hours in a simple defined culture medium. In vivo, spermatozoa may take up to a week to move from the seminiferous tubules in the testes to the cauda epididymis and during the whole of this time they would be vulnerable to RF-EMR exposure [23].

We recognize that these studies were conducted using spermatozoa suspended in a simple defined culture medium rather than the epididymal plasma in which they would be suspended in vivo. Nevertheless the fact that effects on sperm quality have previously been observed in both whole animal radiation experiments[3] and in epidemiological studies of human subjects exposed to various levels of mobile phone radiation [5], [7], [24], emphasizes the biological and clinical relevance of these findings. Moreover, another recent study has found that exposing human spermatozoa to mobile phone radiation for 1 hour leads to significant declines in motility and vitality in concert with an increase in cellular reactive oxygen species generation [25]. The levels of RFEMR exposure were not quantified in this study nor were the sources of ROS identified. Nevertheless, these findings reinforce the general conclusions generated in this paper, particularly with respect to central role played by oxidative stress. The ever-increasing prevalence of mobile communications technology means that humans are now exposed to higher amounts of RF-EMR than ever before. Mobile phones are commonly carried in bags or in pockets in very close proximity to the body. In addition to this, these devices can be stored adjacent to the same part of the body for extended periods of time. In this context, exposure of the male reproductive system to RF-EMR is clearly a significant issue.

The particular significance of the present study is that it not only demonstrates a direct effect of RF-EMR on sperm motility, vitality and DNA integrity but also identifies a potential causative mechanism involving electron leakage from the mitochondrial electron transport chain and the induction of oxidative DNA damage. In part, these mechanistic insights have been achieved because the cell type used in these studies, the human spermatozoon, has an extremely simple cellular architecture, lacking significant cytosol and possessing few cellular organelles other than the sperm nucleus, flagellum and mitochondria. One consequence of this structure is that these cells are uniquely vulnerable to oxidative stress. Moreover, such stress is already known to induce the functional and structural lesions observed in this study including both a loss of motility mediated by peroxidative damage to the sperm plasma membrane, as well as the formation of DNA base adducts in the sperm nucleus that ultimately lead to DNA fragmentation [26], [27].

Notwithstanding the specialized nature of mammalian spermatozoa, the mechanisms suggested by this study may also apply to RF-EMR-mediated damage in other cell types. The RF-EMR used for communications, including mobile phone networks, is not of high enough power to be classed as ionizing radiation. The latter has sufficient energy to pull away electrons, dramatically altering the properties of affected molecules and typically creating extremely reactive radical species. RF-EMR does not contain sufficient energy for these processes. Nevertheless, this form of radiation may have other effects on larger

scale systems such as cells and organelles, which stem from the perturbation of charged molecules and the disruption of electron flow [28], [29]. Mitochondria have one of the largest standing membrane potentials in the body and their energetic functions are entirely dependent on the regulated movement of electrons and protons within the inner mitochondrion membrane. Theoretically, such fluxes might be susceptible to disruptions in local electric fields induced by RF-EMR, offering a potential link between this form of radiation and the non-thermal biological effects observed in this study.

This study clearly demonstrates that RF-EMR can damage sperm function via mechanisms that involve the leakage of electrons from the mitochondria and the creation of oxidative stress. These findings have immediate implications for the high rates of male infertility seen in our species, a majority of which is idiopathic. Furthermore, the fact that sperm DNA is damaged by this form of radiation has additional implications for the health and wellbeing of children born to fathers who have experienced high levels of occupational or environmental exposure to RF-EMR around the time of conception. Overall, these finding raise a number of related health policy and patient management issues that deserve our immediate attention. Specifically we recommend that men of reproductive age who engage in high levels of mobile phone use, do not keep their phones in receiving mode below waist level.

- **Other Sections▼**

Methods

Ethics Statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the University of Newcastle (H-712-0799). All patients provided written informed consent for the collection of samples and subsequent analysis.

Reagents and Solutions

All chemicals and reagents used in this research were obtained from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO) unless stated otherwise. All reagents used were of research grade. All fluorescent probes were purchased from Molecular Probes Inc. (Eugene, OR). Biggers, Whitten and Whittingham (BWW) media supplemented with 1 mg/ml polyvinyl alcohol (PVA) was used in all experiments [30]. It was prepared fresh as required and kept at 37°C with an osmolarity in the range of 290–310 mOsm/kg.

Human spermatozoa

Institutional and State Government ethical approval was secured for the use of human semen samples for this research. The donors were students from the University of Newcastle donor program who had no known prior male reproductive pathologies including varicocele and infection. From this pool, 22 normozoospermic donors were used in this study. The average (±SEM) age of these donors was 24.1±1.1 y. After allowing at least 30 min for liquefaction to occur, spermatozoa were separated from seminal plasma on a discontinuous two-step Percoll gradient, as described [16]. The isolated spermatozoa were washed with 10 ml BWW, centrifuged at 600× g for 15 min and finally resuspended in HEPES-buffered BWW at a concentration of 20×10^6/ml supplemented with 1 mg/ml PVA. After acquiring each sperm fraction, the vitality, motility and cell density of the spermatozoa were evaluated. Vitality was determined by transferring 5 µl of each cell fraction onto a microscope slide followed by the addition of 5 µl of 0.5% eosin; the percentage of non-viable cells staining pink was then assessed by

light microscopy. Motility was assessed by transferring 6 µl of the same sample onto a slide which was then covered with a coverslip and examined by phase contrast microscopy. For both the vitality and motility assessments, 100 cells were counted and the results expressed as a percentage.

Radio Frequency Electromagnetic Radiation and Waveguide

In this study, a cylindrical waveguide copied from the design by Gajda *et al* [31]was constructed such that 1.8 GHz radiation could propagate along the waveguide and also so that 35 mm Petri dishes could be accommodated within the waveguide. To produce the radiation, a 3 GHz function generator (E4431B; Agilent, Palo Alto, CA) was used to generate a pure tone of 1.8 GHz. This signal was amplified by a linear radio-frequency (RF) amplifier and the amplifier output was split and connected through a matching network to antennae in the waveguide. The antenna matching circuit was tuned for maximum energy transfer to the antenna. The waveguide was encased in a brass mesh Faraday cage and the end was filled with 15 cm thick carbon-impregnated foam (RFI Industries, Bayswater, Victoria, Australia), which absorbs RF radiation, minimizing the reflection of radiation back into the waveguide and reducing the RF power by more than 50 dB outside the Faraday cage compared to the power at the amplifier output. A spectrum analyser (Advantest, Tokyo, Japan) connected to a Hameg HZ530 E-field probe (Hameg GmbH, Mainhausen, Germany) was used to check radiation levels and frequency prior to irradiation. The SAR values for the irradiations were calibrated by measuring the temperature rise in saline solution at power levels 20 dB or 100× higher than for the normal irradiations. The calibration procedure is complicated because (i) the saline solution loses heat energy to the surroundings at the same time as it is heated by the RF radiation and (ii) the temperature rise must be measured by an electronic thermometer to achieve the 0.1°C resolution required; however, the RF field interfered with the thermometer operation. As a consequence of these factors, the saline temperature was measured as a function of the time delay after the RF field was turned off and the temperature change extrapolated back to zero delay. Multiple measurements were made for RF irradiation times varying from 15 to 120 s and temperature increases up to 2.2°C above the ambient temperature were measured. After allowing for heat losses to the surroundings, the power level of 38.8 dBm at the amplifier output used in these measurements gave rise to a saline temperature rise of $0.053\pm0.008°Cs^{-1}$, giving a SAR of 220 ± 33 Wkg^{-1}. This error is similar to the variation in SAR observed in reference paper as a function of probe position [31]. The values of SAR reported in this paper were calculated from the above SAR, linearly scaled by the amplifier output power.

Following sperm purification and initial analysis, the high density Percoll fraction was prepared as a 1 ml suspension in BWW containing 5×10^6 cells and transferred into 35 mm Petri dishes. The cells to be irradiated were placed inside the waveguide while the control cells where placed adjacent to the waveguide but outside the Faraday cage. The SAR levels (0.4–27.5 W/kg) were fixed by setting the RF source to the appropriate dBm value. For all RF-EMR exposures (and respective controls) spermatozoa were incubated at room temperature (21°C) for a period of 16 h. Motility and vitality was measured prior to as well as after treatment. ROS and DNA damage assays were completed on both the exposed cells and respective controls after incubation.

Dihydroethidium Assay

Dihydroethidium (DHE) is a poorly fluorescent 2-electron reduction product of ethidium that on oxidation produces DNA sensitive fluorochromes that generate a red nuclear fluorescence when excited at 510 nm. The results obtained with this probe have been validated as a measure of the ability of human spermatozoa to generate ROS, including definitive identification of the superoxide anion [32]. For the assay, DHE and the vitality stain, SYTOX® Green (Molecular Probes), were diluted in BWW/PVA and added to 2×10^6 spermatozoa in a final volume of 200 µl comprising 175 µl of purified sperm suspension, 5 µl of test compound and 20 µl of the DHE:SYTOX® green mixture to give final concentrations of 2 µM DHE and 0.5 µM SYTOX® green. The cells were then incubated in the dark at 37°C for 15 min, washed once (600×*g* for 5 min) and the resultant red and green fluorescence measured on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA), as described [32]. The unstained control displayed 0.09%±0.03% DHE positivity, the DHE positive control (treated with 100 µM arachidonic acid) displayed 99%±1% DHE positivity and the SyG positive control (frozen-thawed cells) displayed 98%±1% SyG positivity. The inclusion of SyG in this assay ensured that the production of ROS was only being assessed in live cells.

MitoSOX Red (MSR) Assay

MSR is a poorly fluorescent compound similar to DHE but carrying a charge that results in the selective accumulation of this probe within the mitochondria. Following reaction with the superoxide anion, MSR produces DNA sensitive fluorochromes that generate a red fluorescence when excited at 510 nm that can be detected by flow cytometry. As with the DHE assay, SyG was used in order to ensure that only live cells were evaluated in this assay. MSR and SyG stock solutions (in DMSO) were diluted in BWW/PVA and 20 µl of each added to each treatment to give final concentrations of 2 µM and 0.05 µM respectively in a final volume of 200 µl. The cells were incubated at 37°C away from light for 15 min, centrifuged at 600× *g*for 5 min and the supernatant discarded. The pellet was then washed in 200 µl BWW/PVA, resuspended in 1 ml of this medium and transferred to 5 ml FACS tubes for analysis by flow cytometry. [15] The unstained control displayed 0.66%±0.32% MSR positivity, the MSR positive control (treated with 100 µM arachidonic acid) displayed 96%±3% MSR positivity and the SyG control displayed 96%±1% SyG positivity.

Assay for 8-hydroxy-2′-deoxyguanosine (8-OH-dG)

The formation of the 8-OH-dG base lesion, which is a biomarker for oxidative stress, was measured using an anti-8-OH-dG antibody (supplied in the Biotrin OxyDNA test Kit, Biotrin International Ltd, Dublin, Ireland) which was conjugated with a fluorescent label, fluorescein isothiocyanate (FITC). The level of FITC fluorescence was then measured using flow cytometry. For the positive control, spermatozoa were incubated for 1 h at room temperature with H_2O_2 (2 mM) and $FeCl_2{\bullet}4H_2O$ (1 mM) in a final volume of 200 µl BWW. The initial H_2O_2concentration was determined by measuring absorbance at 240 nm ($\varepsilon = 43.6\ M^{-1}cm^{-1}$). The cells were then washed twice in BWW, resuspended in 100 µl of 2 mM dithiothreitol (DTT) in BWW and incubated for 45 min at 37°C. After centrifugation at 600× *g* for 5 min, the cells were then fixed by resuspending the pellet in 100 µl Phosphate Buffered Saline (PBS) and 100 µl 4% paraformaldehyde and incubated at 4°C for 15 min. The cells were then washed in PBS and stored in 200 µl 0.1 M glycine at 4°C and stored for a maximum of 1 week. Fixed cells were washed and resuspended in 100 µl 0.2% Triton-X and incubated at room temperature for 15 min. Cells were then

washed in Wash Solution (Biotrin OxyDNA test Kit, Biotrin International Ltd.) and 50 µl blocking solution (Biotrin OxyDNA test Kit, Biotrin International Ltd.) added before incubation at 37°C for 1 h. The anti-8-OH-dG antibody was further purified by adding approximately 1 mg of activated charcoal powder, followed by incubation at room temperature for 1 h and centrifugation at 600× *g* for 5 min. This step was repeated once more for complete removal of the charcoal. The supernatant containing the purified antibody was then added in a 1:50 dilution to the fixed cells in wash solution with a final volume of 100 µl. Finally, cells were washed twice, resuspended in 1 ml PBS and transferred to 5 ml FACS tubes for flow cytometric analysis. The unstained control and positive (H_2O_2/Fe^{2+}) control displayed 0.09%±0.02% and 97%±1% 8-OH-dG positivity, respectively.

TUNEL Assay

Spermatozoa were centrifuged (600× *g* for 4 min) before resuspending the pellet in 100 µl of fresh permeabilization solution (10 mg sodium citrate, 10 µl triton-X in 10 ml dH_2O) and incubating for 2 min at 4°C. The cells were then centrifuged (600× *g* for 4 min) and the pellets washed with PBS. The positive control samples were treated with 100 µl of DNase I (1 mg/ml) for 30 min at 37°C in a humid environment. TUNEL labeling was achieved with the In Situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Cells were then washed twice in PBS, diluted to a final volume of 500 µl in PBS and kept in the dark for analysis using flow cytometry.

Analysis by Flow Cytometry

For flow cytometry analysis, Falcon 35 (2008) 5 mL polystyrene round bottom tubes were used for aspirating the sample into the fluorescence activated cell sorter (FACS). At least 5,000 cells were analyzed for each assay using a FACS™ calibur (Becton Dickinson) and the gates were set, based on forward and side scatter, such that only spermatozoa were assessed [15]. Fluorescence was measured upon excitation by a 15 mW argon-ion laser at 488 nm and was paired with emission measurements using 530/30 band pass (green/FL-1), 585/42 band pass (red/FL-2) and >670 long pass (far red/FL-3) filters. The FL-1 and the FL-2 filters were used for the vitality stain (SyG) and ROS stain (DHE) respectively. For TUNEL and 8-OH-dG analysis, only the FL-1 filter was used and for these assays. The software used to analyze the data was CellQuest Pro (BD Biosciences, San Jose, CA).

Statistics

All experiments were repeated at least 3 times on independent samples and the results analyzed by ANOVA using the SuperANOVA programme (Abacus Concepts Inc, CA) on a MacIntosh G5 computer; post hoc comparison of group means was determined by Fisher's PLSD test. Differences with a P value of <0.05 were regarded as significant. All data are presented as the mean value±SEM.

Footnotes

Competing Interests: The authors have declared that no competing interests exist.
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- **Other Sections▼**

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Male factor

Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study

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Objective
To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen.

Design
Prospective pilot study.

Setting
Center for reproductive medicine laboratory in tertiary hospital setting.

Samples
Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9).

Intervention(s)
After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions.

Main Outcome Measure(s)
Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage.

Result(s)

Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group.
Conclusion(s)
Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.
Key Words: Cell phone radiation; radiofrequency electromagnetic waves; sperm; fertility; reactive oxygen species; oxidative stress; EMW
Article Outline

A.A. has nothing to disclose. N.D. has nothing to disclose. K.M. has nothing to disclose. A.V. has nothing to disclose. R.M. has nothing to disclose. E.S. has nothing to disclose. R.S. has nothing to disclose.
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Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study

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Objective: To investigate the effect of cell phone use on various markers of semen quality.
Design: Observational study.
Setting: Infertility clinic.
Patient(s): Three hundred sixty-one men undergoing infertility evaluation were divided into four groups according to their active cell phone use: group A: no use; group B: <2 h/day; group C: 2–4 h/day; and group D: >4 h/day.
Intervention(s): None.
Main Outcome Measure(s): Sperm parameters (volume, liquefaction time, pH, viscosity, sperm count, motility, viability, and morphology).
Result(s): The comparisons of mean sperm count, motility, viability, and normal morphology among four different cell phone user groups were statistically significant. Mean sperm motility, viability, and normal morphology were significantly different in cell phone user groups within two sperm count groups. The laboratory values of the above four sperm parameters decreased in all four cell phone user groups as the duration of daily exposure to cell phones increased.
Conclusion(s): Use of cell phones decrease the semen quality in men by decreasing the sperm count, motility, viability, and normal morphology. The decrease in sperm parameters was dependent on the duration of daily exposure to cell phones and independent of the initial semen quality. (Fertil Steril® 2008;89:124–8. ©2008 by American Society for Reproductive Medicine.)

Key Words: Cell phone, electromagnetic radiations, sperm parameters, male infertility

Cell phones have become indispensable devices in our daily life. These phones operate between 400 MHz and 2000 MHz frequency bands and emit radiofrequency electromagnetic waves (EMW). Reports of potential adverse effects of radiofrequency EMW on brain, heart, endocrine system, and DNA of humans and animals are widely reported in the literature. Electromagnetic waves alter brain electroencephalographic activity and cause disturbance in sleep (1); cause difficulty in concentration, fatigue, and headache (2); and increase reaction time in a time-dependent manner (3). They increase the resting blood pressure (4) and reduce the production of melatonin (5). They are also implicated in DNA strand breaks (6). However, the concern that cell phone use might have adverse impacts on the semen quality has not been extensively addressed.

Infertility affects approximately 15% of couples of reproductive age, and with nearly half of these cases resulting from male factor infertility this area of research is of great interest to both physicians and research scientists (7, 8). The relationship between cell phone use and male infertility remains unclear. Harmful EMW emitted from cell phones may interfere with normal spermatogenesis and result in a significant decrease in sperm quality. There are two reports available that show an effect of cell phones on sperm motility in humans (9, 10). Animal studies indicate that EMW may have a wide range of damaging effects on the testicular function and male germ line (11, 12). Electromagnetic waves can affect reproductive function through both thermal and nonthermal effects (13).

The objective of the present study was to assess the effects of cell phone use on various sperm parameters among patients undergoing infertility evaluation at a male infertility clinic. Our goal was to better understand the role of cell phone use in male infertility and assess the need for any

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protective measures to prevent harmful effects of EMW, if any, on the male reproductive system.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board, and informed consent was obtained from all patients. In this observational study we examined 361 men attending an infertility clinic from September 2004 to October 2005. The age of the study population was 31.81 ± 6.12 years (mean ± SD). Subjects with a history of smoking, chewing tobacco, alcohol consumption, orchitis, varicocele, tuberculosis, diabetes mellitus, and hypertension were excluded from the study. In addition, patients who suffered from viral/bacterial infection in the past 4 weeks, presented with a history of cardiac, neural, or nephrotic disease, or had a family history of any genetic disease were also excluded.

Semen samples were collected by masturbation in a sterile wide-mouthed calibrated container after an abstinence period of 5 days. Semen analysis was performed according to World Health Organization guidelines to evaluate eight sperm parameters: volume, liquefaction time, pH, viscosity, sperm count, motility, viability, and percentage normal morphology (14). The information on cell phone usage of the patients was recorded and the subjects were divided into 4 groups according to their daily active cell phone usage, i.e., talking time: group A: no use (n = 40); group B: <2 h/day (n = 107); group C: 2–4 h/day (n = 100;); and group D: >4 h/day (n = 114). The technicians analyzing the semen samples were blinded to the use of cell phones by the subjects.

Correlation was determined between eight sperm parameters by Pearson correlation coefficients. Multivariate analysis of covariance (MANCOVA) was used to assess the eight sperm parameters among four groups of cell phone users simultaneously, adjusted by patient age (as covariate). When age as a covariate in the MANCOVA was found to be nonsignificant (F = 0.92; P=.4975), subsequent analysis was done by multivariate analysis of variance (MANOVA). Sperm parameters were transformed to multivariate normals where appropriate before analysis, and results were reported on a back-transformed scale unless otherwise indicated.

Because patients are often grouped as normal or abnormal based on the sperm count, we also assessed if sperm parameters differed among cell phone use groups within sperm count groups. This was accomplished by dividing our study population into two groups: normospermic (≥20 million/mL; n = 297) and oligospermic (<20 million/mL; n = 64). We also reclassified the subjects into two cell phone user groups based on their frequency of active cell phone use: >4 h/day (n = 114) and <4 h/day (n = 247) to use a two-way MANOVA for statistical evaluation. Difference in each sperm parameter between these groups was assessed using Bonferroni simultaneous confidence intervals with a significance level at α=.05. Statistical software packages R (Version 2.3.0; R Foundation for Statistical Computing, Vienna, Austria) and SAS (Version 9.1, SAS Institute, Cary, NC) were used.

RESULTS

A strong correlation was seen between sperm count, motility, viability, normal morphology, and pH; motility and viability were almost perfectly correlated. Semen analysis in the four cell phone user groups showed a decrease in sperm count, motility, viability, and normal morphology with the increase in daily use of cell phone (Table 1; Fig. 1). The difference between cell phone user groups for each sperm parameter was assessed simultaneously using Bonferroni simultaneous confidence intervals (SCI). The 95% Bonferroni SCI for each variable showed that sperm count, percentage motility, viability, and normal morphology differ significantly among most cell phone use groups (Table 2). A significant difference was seen in the sperm parameters motility, viability, and normal morphology among the two sperm count groups (F = 21.86; P<.0001) when evaluated by using two-way MANOVA (Table 3).

DISCUSSION

Currently there are over 700 million cell phone users in the world. These phones operate at different frequencies in

TABLE 1

Semen analysis results in four cell phone use groups (values are mean ± SD).

Parameters	Group A	Group B	Group C	Group D
Volume (mL)	2.86 ± 1.67	3.16 ± 1.62	2.83 ± 1.40	3.37 ± 1.80
Liquefaction time (min)	20.00 ± 3.58	20.04 ± 3.18	20.85 ± 3.56	20.39 ± 4.11
pH	7.67 ± 0.20	7.67 ± 0.18	7.76 ± 0.19	7.78 ± 0.16
Viscosity	3.00 ± 1.01	2.98 ± 1.03	3.11 ± 1.21	2.95 ± 1.14
Sperm count (×10^6/mL)	85.89 ± 35.56	69.03 ± 40.25	58.87 ± 51.92	50.30 ± 41.92
Motility (%)	67.80 ± 6.16	64.57 ± 8.47	54.72 ± 10.97	44.81 ± 16.30
Viability (%)	71.77 ± 6.75	68.21 ± 8.65	57.95 ± 11.28	47.61 ± 16.67
WHO morphology (% normal)	40.32 ± 13.06	31.24 ± 12.24	21.36 ± 10.12	18.40 ± 10.38

Note: Group A: no use (n = 40); group B: <2 h/day (n = 107); group C: 2–4 h/day (n = 100); and group D: >4 h/day (n = 114). Means and SD were based on data on the original scale; all analyses were done with appropriately transformed data.

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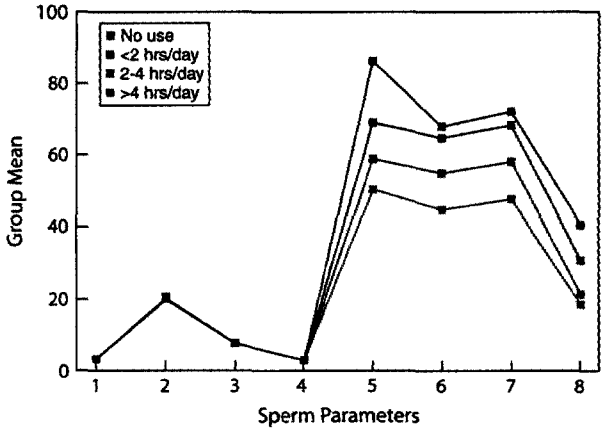
FIGURE 1

Sperm parameter profile for cell phone use groups. The x-axis lists eight sperm parameters: 1 = volume; 2 = liquefaction time; 3 = pH; 4 = viscosity; 5 = sperm count; 6 = motility; 7 = viability; and 8 = percent normal morphology. The y-axis depicts the mean value of the corresponding sperm parameters for each cell phone use group.



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different countries and continents. Exposure of radiofrequency energy depends upon the frequency of the cellular phone. Analog phones operate at 450–900 MHz, digital phones (Global System for Mobile Communications [GSM]) at 850–1900 MHz, and third-generation phones at approximately 2000 MHz (15). For years the cell phone companies have assured people that cell phones are perfectly safe. For assessing exposure from transmitters located near the body, the most useful quantity is the specific absorption rate (SAR), the amount of radiofrequency energy absorbed from the phone into the local tissues. The SAR of cell phones varies from 0.12 to 1.6 W/kg body weight depending upon the model. In the United States, the upper limit of SAR allowed is 1.6 W/kg (16).

We studied the sperm parameters of 361 males attending an infertility clinic after segregating them into four different groups based on their daily active use of cell phone. We found that most of the comparisons of four sperm parameters: sperm count, motility, viability, and normal morphology between all the cell phone user groups were significantly different. This led us to suggest that the use of cell phones may adversely affect the quality of semen by decreasing the sperm counts, motility, viability, and morphology, which might contribute to male infertility. However, these four sperm parameters showed significant positive correlation among each other. Therefore, the decrease in value of one sperm parameter is bound to reduce the other parameter also. Another significant finding of our study is the decline in the quality of semen based on the active cell phone usage time. The laboratory values of the four sperm parameters were lower in the

TABLE 2

Simultaneous confidence intervals of differences between cell phone use groups evaluating eight sperm parameters.

Parameters	Groups A & B	Groups A & C	Groups A & D	Groups B & C	Groups B & D	Groups C & D
Volume (mL)	−0.091 to 0.072	−0.084 to 0.083	−0.102 to 0.058	−0.389 to 0.053	−0.046 to 0.040	−0.063 to 0.026
Liquefaction time (min)	−2.27 to 2.18	−3.10 to 1.40	−2.60 to 1.87	−2.47 to 0.87	−1.96 to 1.27	−1.19 to 2.10
pH	−0.115 to 0.105	−0.209 to 0.014	−0.223 to −0.004	−0.175 to −0.009[a]	−0.189 to −0.02[a]	−0.098 to −0.065
Viscosity	−0.66 to 0.70	−0.80 to 0.58	−0.63 to 0.72	−0.64 to 0.38	−0.47 to 0.52	−0.35 to 0.66
Sperm count ($\times 10^6$/mL)	−1.67 to 4.40	1.29 to 7.49[a]	4.05 to 10.03[a]	−0.85 to 2.57	0.60 to 3.81[a]	−1.35 to 1.97
Motility (%)	−6.16 to 45.83	12.86 to 65.11[a]	22.71 to 74.49[a]	11.04 to 56.09[a]	22.21 to 66.52[a]	6.65 to 51.36[a]
Viability (%)	−5.55 to 48.84	14.04 to 68. 70[a]	24.42 to 78.59[a]	11.69 to 58.82[a]	23.55 to 69.92[a]	7.29 to 54.07[a]
WHO morphology (% normal)	0.12 to 1.11[a]	2.65 to 3.66[a]	4.14 to 5.12[a]	0.70 to 1.26[a]	1.60 to 2.12[a]	−0.12 to 0.41

Note: Group A: no use (n = 40); group B: <2 h/day (n = 107); group C: 2–4 h/day (n = 100); and group D: >4 h/day (n = 114). Means and SD were based on data on the original scale; all analyses were done with appropriately transformed data.

[a] Significant ($P<.05$) using multivariate analysis of variance and Bonferroni simultaneous confidence intervals.

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TABLE 3

Difference between two sperm count groups within cell phone use groups evaluating seven sperm parameters.

Sperm Parameters	Group 1,[a] mean ± SD	Group 2,[b] mean ± SD	Simultaneous confidence intervals of difference between groups 1 and 2
Volume (mL)	2.75 ± 1.57	3.17 ± 1.64	−0.078 to 0.029
Liquefaction time (min)	20.39 ± 3.81	20.37 ± 3.61	−1.85 to 1.85
pH	7.80 ± 0.17	7.71 ± 0.18	−0.01 to 0.15
Viscosity	2.90 ± 1.43	3.03 ± 1.03	−0.72 to 0.49
Motility (%)	42.00 ± 17.16	58.96 ± 12.35	−59.49 to −10.31[c]
Viability (%)	44.62 ± 17.47	62.41 ± 12.77	−62.58 to −11.45 [c]
WHO morphology (% normal)	14.98 ± 9.11	27.71 ± 13.11	−1.69 to −1.05[c]

Note: Means and SD were based on data on the original scale; all analyses were done with appropriately transformed data.
[a] Group 1: sperm count: 9.26 ± 5.54 × 10^6/mL (n = 64).
[b] Group 2: sperm count: 73.57 ± 41.57 × 10^6/mL (n = 297).
[c] Significant ($P<.05$) using two-way MANOVA and Bonferroni simultaneous confidence intervals.

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group using cell phones for longer periods of time. When we tried to evaluate the effects of cell phone use within two different sperm count groups (normospermic and oligospermic), we found that the sperm motility, viability, and morphology were still significantly different in subjects using cell phone for less than 4 h/day than those who were using it more. Our initial data have led us to believe that the effect of cell phone use on sperm parameters do not depend on the initial semen quality of the subjects.

In a recent study done by Fejes et al. (9) on 371 men undergoing infertility evaluations, the duration of possession and the daily transmission times of cell phones correlated negatively with the proportion of rapid progressive motile sperm and positively with the proportion of slow progressive motile sperm, although there were no changes in the total motility. Therefore they concluded that prolonged use of cell phones might have negative effects on sperm motility. Davoudi et al. (10), in a prospective study involving 13 men with normal semen analysis, also found that using GSM phones for 6 h/day for 5 days decreased the rapid progressive motility of sperm. The present results are in accordance with these authors, although we found that not only motility but also sperm count, viability, and morphology are negatively affected by the use of cell phones.

In their study on mice, Aitken et al. (12) suggested that radiofrequency EMW might have a genotoxic effect on epididymal spermatozoa, which needs further investigation (12). Contrary to this, Malyapa et al. (17) were unable to find any damaging effects of Code Division Multiple Access phones, with frequency modulation 847.74 MHz, on mouse fibroblasts and human glioblastoma cells. Dasdag et al. (18) also failed to report any adverse effect of cell phone exposure on sperm count, morphology, and histologic structure of testis in rats. However, it is impractical to compare a rat model to humans because of its small testicular size, nonpendulous scrotum, and the fact that its testis can migrate between the abdomen and scrotum in the inguinal canal (19).

Although the present study suggests the role of cell phones in male infertility, the mechanism of action of EMW emitted from cell phones on male reproductive system is still unclear. Electromagnetic waves can possibly affect reproductive function via three mechanisms: 1) an EMW-specific effect; 2) a thermal molecular effect; or 3) a combination of these (13). Wang et al. (20) suggested in their study on mice that Leydig cells are among the most susceptible cells to EMW, and injury to Leydig cells may affect spermatogenesis. Increase in tissue or body temperature on exposure to EMW may also cause reversible disruption of spermatogenesis (21–23). Electromagnetic wave–dependent decrease in melatonin (5) an antioxidant, can predispose sperm to oxidative stress. Because a negative correlation is seen between sperm motility and sperm chromatin damage (24), and EMW have been shown to effect sperm motility, another possible mechanism of effects of EMW on sperm is DNA damage. Further research is needed to identify the mechanism of action of EMW emitted from cell phones on the male reproductive system.

The present study has a few limitations. We relied only on the self-perceived history of the subjects and did not validate their cell phone use. We did not take into account the occupational history of the subjects and EMW exposure from other sources such as radiotowers, PDAs, Bluetooth devices, computers, etc. We also did not consider the effects of cell phone possession in standby position. Inability to analyze covariates other than age is also a limiting factor. Because each cell phone model has a different specific absorption rate, differentiating between the effects of various models is also important. We are trying to address these issues in a follow-up

study. Nevertheless, the present study has revealed significant findings which pave way for future research in this area.

In conclusion, our results suggest that the use of cell phones by men is associated with a decrease in semen quality. The decrease in sperm count, motility, viability, and normal morphology is related to the duration of exposure to cell phones. These effects may not depend on the initial semen quality of the subjects. More studies are needed to identify the mechanism involved in the reduction of semen quality.

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